



AMENDMENTS TO THE SPECIFICATION

1) Please amend the paragraph located at page 26, lines 10-14 as follows:

Q1 C. 9- [N- [2- (5,5-dimethyl- [1,3,2]dioxaborinan-2-yl)benzyl] - N- [3- (methacrylamido)propylamino]methyl] -10- [N- [2- (5,5-dimethyl- [1,3,2]dioxaborinan-2-yl)benzyl] -N- [2- (2-hydroxyethoxy) - ethylamino]methyl]anthracene. (Single-methacrylamide monomer).

2) Please amend the paragraph located at page 29, lines 1-2 as follows:

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Q2 B. 9,10-bis[N- [2- (5,5-dimethyl- [1,3,2]dioxaborinan-2-yl)benzyl] - N- [3- (methacrylamido)propylamino]methylantracene.

3) Please amend the paragraph located at page 29, line 24 to page 30, line 17 as follows:

Q3 A solution of N,N-dimethylacrylamide (40% wt.) and N,N'-methylenebisacrylamide (0.8% wt.) in ethylene glycol was prepared. 9,10-bis[N- [2- (5,5-dimethyl- [1,3,2]dioxaborinan-2-yl) -benzyl] -N- [3- (methacrylamido) -propylamino]methylantracene (17.8 mg,  $2 \times 10^{-5}$  mole) and 40  $\mu$ L of aqueous ammonium persulfate (5% wt) were combined with 1 mL of ethylene glycol monomer

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solution. The resulting solution was placed in a glove box purged with nitrogen. An aqueous solution of N,N,N',N'-tetramethylethylenediamine (80  $\mu$ L, 5% wt.) was added to the monomer formulation to accelerate polymerization. The resulting formulation was poured in a mold constructed from microscope slides and 100 micron stainless steel spacer. After being kept for 8 hours in nitrogen atmosphere the mold was placed in phosphate buffered saline (PBS) (10 mM PBS, pH=7.4), the microscope slides were separated, and the hydrogel was removed. The hydrogel was washed with 100 mL of PBS containing 1 mM lauryl sulfate sodium salt and 1 mM EDTA sodium salt for 3 days, the solution being changed every day, followed by washing with DMF/PBS (10/90 by vol., 3 x 100 mL), and finally with PBS (pH=7.4, 3 x 100 mL). The resulting hydrogel polymer was stored in PBS (10 mM PBS, pH=7.4) containing 0.2% wt. sodium azide and 1 mM EDTA sodium salt.

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4) Please amend the paragraph located at page 32, lines 12-13 as follows:

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B. 9,10-bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)benzyl]-N-[2-(2-hydroxyethoxy)ethylamino)methyl]anthracene.

5) Please amend the paragraph located at page 33,  
lines 14-18 as follows:

05 C. 9- [N- [2- (5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)benzyl] -N- [2-  
(2-methacroyloxyethoxy)ethylamino]methyl] -10- [N- [2- (5,5-dimethyl-  
[1,3,2]dioxaborinan-2-yl)benzyl] -N- [2- (2-hydroxyethoxy) -  
ethylamino]methyl]anthracene. (Single-methacrylate monomer).

6) Please amend the paragraph located at page 33, line  
19 to page 34, line 10 as follows:

all A solution of 9,10-bis[N- [2- (5,5-dimethyl-  
[1,3,2]dioxaborinan-2-yl)benzyl] -N- [2- (2-hydroxyethoxy) -  
ethylamino]methyl]-anthracene (0.298 g, 0.359 mmole), methacrylic  
acid (0.304 g, 0.300 mL, 3.53 mmole, 9.84 equiv.), DCC (0.965 g,  
4.68 mmole, 13.0 equiv.) and N,N-dimethylamino-pyridine (0.020  
g, 0.16 mmole, 0.46 equiv.) in 15 mL CH<sub>2</sub>Cl<sub>2</sub> at 23°C was stirred  
in the dark for 4 hours. At this time, the reaction mixture was  
filtered and concentrated by rotary evaporation. The residue was  
purified by alumina column chromatography (50 g activated neutral  
alumina, 0-4% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>) to yield 0.150 g (47%) of a yellow  
solid.

7) Please amend the paragraph located at page 34,  
lines 19-23 as follows:

97 D. Water soluble copolymer of 9-[N-[2-(5,5-dimethyl-  
[1,3,2]dioxaborinan-2-yl)benzyl]-N-[2-(2-methacroyloxy-  
ethoxy)ethylamino]methyl]-10-[N-[2-(5,5-dimethyl-  
[1,3,2]dioxaborinan-2-yl)benzyl]-N-[2-(2-  
hydroxyethoxy)ethylamino]methyl]-anthracene and TMAMA (1:50 molar  
ratio).

8) Please amend the paragraph located at page ~~34~~, line  
24 to page 35, line 15 as follows:

98 To a solution of [2-(methacryloxy)ethyl]trimethyl-  
ammonium chloride (TMAMA, 70 wt % aqueous solution, 0.344 g  
monomer, 1.66 mmole, 50 equiv.) in 0.600 mL water was added a  
solution of 9-[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-  
yl)benzyl]-N-[2-(2-methacroyloxyethoxy)ethylamino]-methyl]-10-[N-  
[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)benzyl]-N-[2-(2-  
hydroxyethoxy)ethylamino]methyl]anthracene (0.029 g, 0.033 mmole)  
in 3.00 mL MeOH. To this mixture was added 4,4'-azobis(4-  
cyanovaleric acid) (0.0075 g, 0.027 mmole, 1.6 mole % of total  
monomer). The solution was filtered through a 0.45  $\mu$  membrane  
filter, was purged with nitrogen gas and then heated in the dark

at 55°C for 16 hours. At this time, the viscous solution was cooled to 25°C and concentrated *in vacuo*. The residue was diluted with 20 mL water and filtered through a 0.2  $\mu$  membrane filter. The polymer solution was dialyzed through a cellulose acetate membrane (MWCO 3500) against 2 x 4 L of water. From the dialysis was obtained 38.5 mL of polymer solution. Concentration of a portion of this solution to dryness indicated 0.0075g polymer per 1.0 mL solution. Overall 0.289g (77%) yield of polymer.

9) Please amend the paragraph located at page 36, lines 1-3 as follows:

F. 9,10-bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)benzyl]-N-[2-(2-methacroyloxyethoxy)ethylamino]-methyl]anthracene. (Dual-methacrylate monomer).

10) Please amend the paragraph located at page 36, lines 4-18 as follows:

A solution of 9,10-bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)benzyl]-N-[2-(2-hydroxyethoxy)ethylamino]methyl]-anthracene (0.100 g, 0.120

Q10 mmole), methacrylic acid (0.112 g, 0.110 mL, 1.30 mmole, 10.8 equiv.), DCC (0.316 g, 1.53 mmole, 12.8 equiv.) and N,N-dimethylamino-pyridine (0.014 g, 0.11 mmole, 0.92 equiv.) in 5 mL CH<sub>2</sub>Cl<sub>2</sub> was stirred at 0°C for 1 hour, then 23°C for 22 hours. At this time, the reaction mixture was filtered and concentrated by rotary evaporation. The residue was purified by alumina column chromatography (30 g activated neutral alumina, 0-2% CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>) to yield 0.030 g (26%) of a yellow solid. This product may be copolymerized with one or more other monomers to form an indicator macromolecule. The boronate groups should be deprotected prior to use.

11) Please amend the paragraph located at page 37, line 11 to page 38, line 8 as follows:

Q11 A solution of hydroxyethyl methacrylate (HEMA, 0.078 mL, 0.084 g, 0.64 mmol), methacrylic acid (MAA, 0.030 mL, 0.030 g, 0.35 mmol), polyethyleneglycol dimethacrylate 1000 (PEGDMA, 0.5 mg/mL aqueous solution, 0.048 mL), and N,N-dimethyl-N-methacryloxyethyl-N-(3-sulfopropyl)-ammonium-batain (SPE, 0.462 g, 1.65 mmol) in 0.900 mL of ethylene glycol was prepared. 9,10-bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)benzyl]-N-[2-(2-methacroyloxyethoxy)ethylamino]-methyl]anthracene (0.0096g, 0.010

mmol) and 0.020 mL of 5% wt. aqueous solution of ammonium persulfate were combined with 0.500 mL of the ethylene glycol monomer solution. This solution was placed in glove box purged with nitrogen. An aqueous solution of N,N,N',N'-tetramethylethylenediamine (0.040 mL, 5% wt.) was added to the monomer formulation to accelerate polymerization. The resulting formulation was poured in a mold constructed from microscope slides and a 100 micron stainless steel spacer. After being kept for 8 hours in nitrogen atmosphere the mold was placed in phosphate buffered saline (PBS, pH=7.4), the microscope slides were separated, and the hydrogel was removed. The hydrogel was washed with 100 mL of PBS containing 1 mM lauryl sulfate sodium salt for 3 days, the solution being changed every day, followed by washing with MeOH/PBS (20/80 by vol. 3 x 100 mL), and finally with PBS (3 X 100 ML). The resulting hydrogel polymer was stored in PBS (pH=7.4) containing 0.2% wt. sodium azide and 1 mM EDTA sodium salt.

12) Please amend the paragraph located at page 39, lines 2-7 as follows:

Effect of glucose or lactate on acrylamide gel containing  
N-[3-(methacrylamido)propyl]-3,4-dihydroxy-9,10-dioxo-2-

anthracenesulfonamide (Alizarin Red S monomer) and

$\alpha, \alpha'$ -bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)benzyl]-

N-[3-(methacrylamido)propylamino]-1,4-xylene (bis boronic acid monomer):

Q12 [ 13) Please amend the paragraph located at page 41, lines 1-2 as follows:

Q13 D.  $\alpha, \alpha'$ -bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-benzyl]-N-[3-(methacrylamido)propylamino]-1,4-xylene.

[ 14) Please amend the paragraph located at page 42, lines 1-5 as follows:

E. Preparation of acrylamide gel containing

Q14 N-[3-(methacrylamido)propyl]-3,4-dihydroxy-9,10-dioxo-2-

anthracenesulfonamide (Alizarin Red S monomer) and

$\alpha, \alpha'$ -bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-

benzyl]-N-[3-(methacrylamido)propylamino]-1,4-xylene:

[ 15) Please amend the paragraph located at page 42, lines 6-32 as follows:



Q15

Ethylene glycol solution containing 30% wt. acrylamide and 0.8% wt. N,N'-methylenebisacrylamide was prepared. N-[3-(methacrylamido)propyl]-3,4-dihydroxy-9,10-dioxo-2-anthracenesulfonamide (1.5 mg,  $3.38 \times 10^{-6}$  mole) and  $\alpha,\alpha'$ -bis[N-[2-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-benzyl]-N-[3-(methacrylamido)propylamino]-1,4-xylene (28 mg,  $3.54 \times 10^{-5}$  mole) were combined with 800  $\mu$ L of ethylene glycol monomer solution and 40  $\mu$ L of 5% wt. aqueous ammonium persulfate. This formulation was placed in a glove box purged with nitrogen along with a mold constructed from glass microscope slides and 100 micron stainless steel spacer. An aqueous solution of N,N,N',N'-tetramethylethylenediamine (40  $\mu$ L, 5% wt.) was added to the monomer solution to accelerate polymerization and the final formulation was poured into a glass mold. The mold was left under nitrogen atmosphere for 16 hours, after which it was immersed in PBS (pH=7.4) and the glass slides were separated to afford a hydrogel polymer in a form of a thin film. The resulting hydrogel thin film was washed with 100 mL of phosphate buffered saline containing 1 mM lauryl sulfate sodium salt for 3 days, the solution being changed every day, followed by washing with MeOH/PBS (20/80 by vol., 3 x 100 mL), and finally with PBS (pH=7.4, 3 x 100 mL). Hydrogel polymer was stored in PBS (10 mM PBS, pH=7.4) containing 0.2% wt. sodium azide and 1 mM EDTA sodium salt.